

RESEARCH ARTICLE

Spastic paraplegia preceding *PSEN1*-related familial Alzheimer's disease

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Abstract

Introduction: We investigated the frequency, neuropathology, and phenotypic characteristics of spastic paraplegia (SP) that precedes dementia in presenilin 1 (*PSEN1*) related familial Alzheimer's disease (AD).

Methods: We performed whole exome sequencing (WES) in 60 probands with hereditary spastic paraplegia (HSP) phenotype that was negative for variants in known HSP-related genes. Where *PSEN1* mutation was identified, brain biopsy was performed. We

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investigated the link between HSP and AD with *PSEN1* in silico pathway analysis and measured *in vivo* the stability of *PSEN1* mutant γ -secretase.

Results: We identified a *PSEN1* variant (p.Thr291Pro) in an individual presenting with pure SP at 30 years of age. Three years later, SP was associated with severe, fast cognitive decline and amyloid deposition with diffuse cortical plaques on brain biopsy. Biochemical analysis of p.Thr291Pro *PSEN1* revealed that although the mutation does not alter active γ -secretase reconstitution, it destabilizes γ -secretase-amyloid precursor protein (APP)/amyloid beta ($A\beta$) interactions during proteolysis, enhancing the production of longer $A\beta$ peptides. We then extended our analysis to all 226 *PSEN1* pathogenic variants reported and show that 7.5% were associated with pure SP onset followed by cognitive decline later in the disease. We found that *PSEN1* cases manifesting initially as SP have a later age of onset, are associated with mutations located beyond codon 200, and showed larger diffuse, cored plaques, amyloid-ring arteries, and severe CAA.

Discussion: We show that pure SP can precede dementia onset in *PSEN1*-related familial AD. We recommend *PSEN1* genetic testing in patients presenting with SP with no variants in known HSP-related genes, particularly when associated with a family history of cognitive decline.

KEYWORDS

Alzheimer's disease, dementia, hereditary spastic paraplegia, HSP, presenilin, *PSEN1*, spastic gait, spastic paraparesis, spastic paraplegia

1 | BACKGROUND

Alzheimer's disease (AD) is the major cause of dementia worldwide, presenting with memory loss and behavioral disturbances as the core clinical features.¹ Around 4.7 million individuals 65 years of age or older are estimated to carry a diagnosis of AD dementia in the United States alone.² By 2050, AD cases are expected to reach up to 13.8 million in the United States and 130 million worldwide.³ Neuropathologically, intracellular neurofibrillary tangles of abnormally phosphorylated tau protein and extracellular amyloid beta ($A\beta$) plaques are the hallmarks of AD.⁴ The majority of AD cases are sporadic, with a late onset (≥ 65 years). Mutations in presenilin 1 (*PSEN1*) (OMIM: 104311), presenilin 2 (*PSEN2*) (OMIM: 600759) and the amyloid precursor protein *APP* (OMIM: 104760) cause familial forms with early onset disease.^{1,4,5} Familial AD is characterized by memory and behavioral disturbances similar to sporadic AD. Nevertheless, atypical phenotypes associated with spastic paraplegia (SP) have been described in familial AD (FAD), especially in *PSEN1* mutations, the most common genetic cause of AD.^{1,4-8}

1. **Systematic review:** The authors reviewed the literature using traditional (eg, PubMed) sources, meeting abstracts, and presentations. Analysis of all 226 *PSEN1* pathogenic variants reported to date showed that 7.5% presented as pure spastic paraplegia (SP)

followed by cognitive decline, and *PSEN1* cases manifesting initially as SP have a later age of onset and are associated with mutations located beyond codon 200. These mutations were associated with cortical cotton wool plaques (CWP) on neuropathological examination.

2. **Interpretation:** We show that pure spastic paraplegia can precede the onset of dementia in *PSEN1*-related familial Alzheimer's disease (AD) and that clinical features of SP correlate with the position of pathogenic mutations. We recommend *PSEN1* genetic testing in patients presenting with SP negative for variants in known hereditary SP (HSP)-related genes, particularly in the presence of a family history of cognitive decline.
3. **Future directions:** This study highlights the clinical overlap between spastic paraplegia and *PSEN1*-related Alzheimer's disease. Early screening of *PSEN1* mutations would alleviate the need for further invasive testing, such as brain biopsy. Unraveling the phenotypic heterogeneity within *PSEN1* mutation carriers could provide new potential targets for clinical trials of patients with familial AD.

Spastic paraplegia (or SP) is a common feature of many genetic neurological diseases. It is the defining clinical feature of a group of diseases collectively known as hereditary spastic paraplegias (HSPs).^{9,10} HSP can present as pure (defined by isolated lower limb spasticity) or complicated by other neurological manifestations such as cognitive

impairment, ataxia, or peripheral neuropathy.¹¹ All types of inheritance have been reported in HSPs.

In 1997, mutations in *PSEN1* were originally identified as a cause of FAD complicated by SP.^{12–14} *PSEN1*, *PSEN2*, and *APP* encode the catalytic subunit—presenilin1/2 (PSEN1/2)—of the γ -secretase protease complex and its substrate, the amyloid precursor protein (APP). γ -Secretase performs sequential cleavages on the transmembrane domain of APP and many other type 1 transmembrane proteins (eg, LIN-12/Notch proteins).¹⁵ The sequential γ -secretase-mediated processing of APP generates amyloid beta (A β) peptides of different lengths and has been studied in detail.^{8,15} Several reports suggest that other γ -secretase substrates are processed by the protease in a sequential manner, but the precise number and position of cuts are not known.¹⁵

We have reported previously that *PSEN1* AD-linked mutations consistently lead to the release of relatively longer A β s.^{16,17} It is important to note that the enhanced release of longer peptides is caused by the destabilization of γ -secretase-APP interactions during its sequential processing.¹⁸ Of note, the degree of destabilization of the enzyme-substrate complexes had been shown to correlate with the age at AD onset.¹⁸

The AD-linked *PSEN1* mutations associated with SP share characteristic neuropathological findings; one that is shared by all cases is cotton wool plaques (CWPs). CWPs, not typically observed in sporadic AD, represent large non-cored plaques without significant neuritic dystrophy.^{19,20} An expanding number of families with AD complicated by SP has been subsequently reported, many of them displaying characteristic CWP.¹³ Very rarely, SP can even precede the development of cognitive decline in these patients, sometimes by several years.¹³ In the era of next-generation DNA sequencing and emerging disease-modifying therapies for familial and sporadic AD, early provision of a definite genetic diagnosis for these patients becomes extremely important.

Here, we investigated the frequency, neuropathological findings, and phenotypic characteristics of spastic paraplegia (SP) that precedes the onset of dementia in *PSEN1*-related familial AD.

2 | METHODS

2.1 | Subjects

In this retrospective study we recruited 60 Greek HSP index cases referred to a tertiary neurology department under ethics-approved research protocols (UCLH:04/N034) with informed consent. The probands had slowly progressive SP as the first or most prominent manifestation. Acquired and metabolic causes of SP were excluded with extensive autoimmune, metabolic, and neuroimaging investigations, including brain and spine magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) analysis, evoked potentials studies, nerve conduction and electromyography (EMG), electroencephalography (EEG), and testing for HIV, syphilis, serum vitamin B12, and vitamin E.

2.2 | Clinical phenotyping

Comprehensive clinical phenotyping was performed by neurogenetics specialists in Athens (Neurogenetics Unit, UOA) during the period from 2012 to 2019. The Ashworth scale was used to quantify the grade of spasticity, and the Spastic Paraplegia Rating Scale (SPRS) to evaluate the severity of SP. The Montreal Cognitive Assessment (MoCA) was used for cognitive assessment.

2.3 | Neuropathology

Biopsy specimens were fixed overnight in formalin (4%) and routinely processed into liquid paraffin. The sections were cut at 4 μ m thickness with a microtome (Microm, Heidelberg, Germany) and stained with hematoxylin and eosin (H&E) and Congo red. Immunohistochemical staining was performed using an automated staining apparatus (Ventana Benchmark, Roche, Mannheim, Germany) and an antibody directed against A β (Clone 4G8; Covance, Emeryville, California, USA) according to the manufacturer's protocol.

2.4 | Genetic analysis

DNA was extracted from peripheral blood. All cases were investigated with whole exome sequencing (WES). WES and alignments were carried out at Macrogen (Macrogen Inc, Seoul, South Korea). Exome capture was performed using SureSelect Human All Exon V4 enrichment kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced on an Illumina HiSeq4000 instrument (Illumina, San Diego, CA, USA) generating 100-bp paired-end reads. Reads alignment using the hg19 human genome reference, variant calling and annotation were performed as described previously.²¹ All cases were screened and were negative for known HSP-related genes.

The analysis was then extended to include all coding genes. Only variants with MAF of < 0.01 in 1000 Genomes Project,²² NHLBI GO Exome Sequencing, and gnomAD²³ and predicted to be likely pathogenic or known pathogenic, in genes associated with neurological phenotype, were considered as likely causal variants. The *PSEN1* variant was confirmed using bi-directional Sanger Sequencing using the following primers: 5'-GGAAGACTGGCGATTGTGT-3' and 5'-CAGTGACCCTGAAAAATCAAGA-3'.

2.5 | Generation of stable cell lines

Mouse embryonic fibroblasts (MEFs) stably expressing wild-type or the p.Thr291Pro human *PSEN1* were generated as previously reported.²⁴ Briefly, *PSEN1*/*PSEN2* dKO MEFs were cultured in Dulbecco's modified Eagle's medium (DMEM)/F-12 (Life Technologies) containing 10% fetal bovine serum (FBS) (Life Technologies). The cells were transduced with retroviruses, carrying pMSCVpuro plasmids encoding wild-type

(WT) or p.Thr291Pro *PSEN1*, using a replication-defective recombinant retroviral expression system (Clontech). Post transduction the culture medium was supplemented with 5 $\mu\text{g}/\text{mL}$ puromycin (Sigma-Aldrich) to select clones stably expressing PSEN, which were further maintained in DMEM/F-12, 10% FBS, and 3 $\mu\text{g}/\text{mL}$ puromycin. NCT and PSEN1 expression levels and the reconstitution of the functional GSEC complexes in the different cell lines were analyzed by SDS-PAGE/western blot. GSEC inhibitor X (565771) was purchased from Calbiochem and anti-NCT (612290) from BD Biosciences.

The following antibodies were used in the study: anti-FLAG M2 (F3165) from Sigma-Aldrich, anti-PSEN-CTF (MAB5232) from Merck, horseradish peroxidase (HRP)-conjugated anti-mouse (1721011), anti-rabbit IgG (1721019) from Bio-Rad, and anti-PEN-2 (B126.2).²⁵

2.6 | In vitro thermo-activity assays using solubilized γ -secretase

MEF microsomal fractions were solubilized in 1% CHAPSO as described previously.¹⁶ A total of 5 μg of the solubilized material was used in *in vitro* thermo-activity assays with purified APPC99-3xFLAG (1.5 μM final) (as described¹⁸) in 50 mM PIPES, pH 7.0, 0.25 M sucrose, 1 mM EGTA, 1x proteinase inhibitors (Roche), 0.1% phosphatidylcholine, and 2.5% DMSO (or 10 μM Inhibitor X). The activity assays were performed over a temperature gradient ranging from 37°C to 65°C (similar to¹⁸). Briefly, enzyme mixes (containing all components except the substrate) and substrate dilutions were pre-incubated separately at the indicated temperatures for 10 minutes. After pre-incubation, the substrate was added to the enzyme mix and proteolysis proceeded for 4 hours. AICD production was assessed by western blot analysis with M2 FLAG antibody, and A β generation was quantified by MSD multiplex ELISA as described previously.¹⁸ The ELISA capture antibodies (JRF ABO38 for A β 38, JRF/cAb40/28 for A β 40 and JRF/cAb42/26 for A β 42) and the detection antibody (JRF/AbN/25 raised against the N-terminus of A β) were obtained through collaboration with Janssen Pharmaceutica NV (Beerse, Belgium).

2.7 | Analysis of all reported PSEN1 pathogenic variants

We expanded our analysis into the link between SP and *PSEN1*-related AD by investigating the frequency of SP in cases with previously reported *PSEN1* pathogenic variants. Literature searches were conducted on MEDLINE (PubMed), Scopus, clinicaltrials.gov, and database of AD mutations (<http://www.molgen.ua.ac.be/ADMutations>) using several key words and their combinations, up to December 2019. Search terms included: hereditary spastic paraplegia, spastic paraplegia, familial Alzheimer's disease, and *PSEN1*. References from the selected articles were also thoroughly screened for other pertinent articles. Additional searches were performed for specific topics (eg, neuropathology of Alzheimer's disease, cotton wool plaques). Studies reporting mutations in *PSEN1*, presenting initially as SP, were further

analyzed. During the screening of the abstracts/full texts, the publications that were not relevant to this review were removed. No language or publication period restrictions were applied to the initial searches. However, only English-speaking publications were further studied in detail.

2.8 | Pathway analysis

We assessed the biological functions of HSP-causative genes as members of a putative interactome, that is, a protein network jointly mediating several intracellular processes. Expanded details of functional enrichment analyses and *in silico* knockdown of PSEN1 are described in [Supplementary e-1](#).

3 | RESULTS

3.1 | PSEN1 p.Thr291Pro variant presenting with spastic paraplegia several years before the onset of cognitive decline

From screening a cohort of 60 patients with symptoms of HSP we identified one individual presenting with pure HSP phenotype followed by the development of severe cognitive decline several years later. The patient in his thirties (individual II-1, [Figure 1A](#)) with an unremarkable past medical history presented with a 1-year history of progressive gait disturbance. Initial neurological examination revealed a mild spastic gait with pyramidal syndrome (brisk reflexes, bilateral upgoing plantar reflexes, ankle clonus, and increased tone with an Ashworth score of 1 [of 4]). Cognitive function was within normal limits on initial examination (Montreal Cognitive Assessment [MoCA] 30/30). Brain and spine MRI in the proband revealed no specific findings. His mother had an onset of SP in her thirties and no cognitive impairment reported at onset. She became bedridden with severe cognitive impairment and died before age 40. No further family history was available, because she was adopted.

HSP gene panel and multiplex ligation-dependent probe amplification (MLPA) for - copy number variants in *SPG4*, the most frequent cause of pure HSP, were negative. The spasticity progressed, and at 1-year follow-up the patient presented with a severe spastic gait but no additional clinical features. Memory disturbances were first noted at ≈ 2.5 years following the onset of spasticity and prompted a re-evaluation of the initial diagnosis. A repeat brain MRI did not show any abnormal findings; therefore, a brain biopsy was performed to exclude a spongiform encephalopathy. Of interest, the only findings on brain biopsy were A β -A4-deposition within the vessel walls with several diffuse cortical CWP. Three years following symptom onset, he had severe worsening of his cognitive abilities (MoCA20/30, MMSE25/30) with further motor deterioration. At last examination, 4.5 years into the disease (at 37.5 years), the patient was wheelchair-bound with severe SP, walking only a few steps with bilateral support. SP rating scale was 48/52 and MoCA was 14/30.

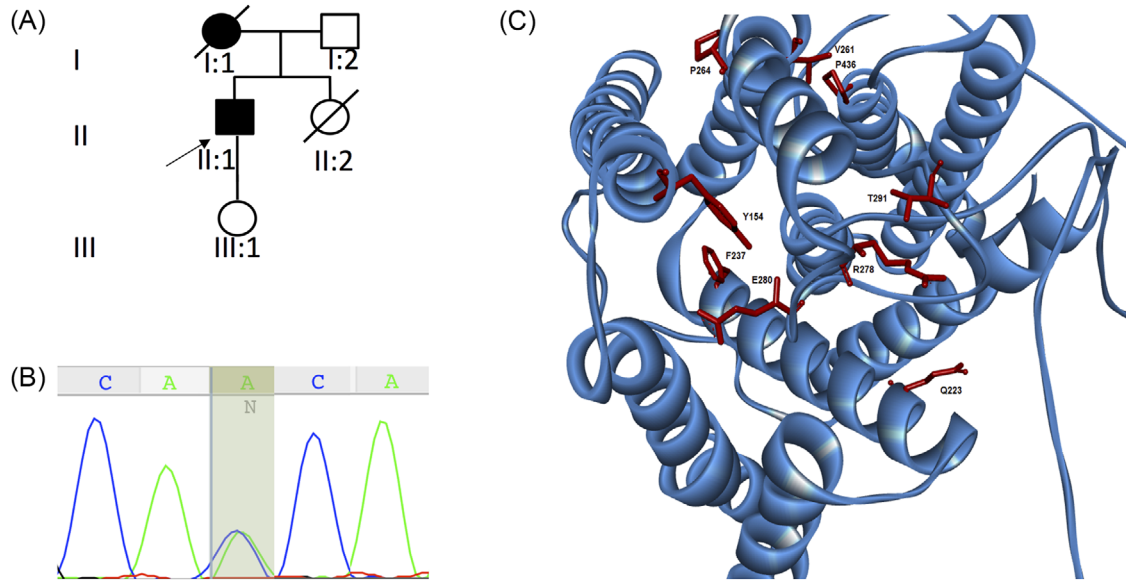


FIGURE 1 Genetic and protein analysis for the *PSEN1* mutation-associated spastic paraplegia. A, Pedigree of the family reported in this study. B, Sanger sequencing confirmation of the proband highlighting the c.871A > C (p.Thr291Pro) mutation. C, Localization of *PSEN1* mutations associated with spastic paraparesis as presenting phenotype. *PSEN1* protein has nine transmembrane domains. Pathogenic substitutes in the p.Thr291Pro and other reported mutations are shown

WES in the proband (II-1) revealed the heterozygous variant c.871A > C (p.Thr291Pro) in *PSEN1* (NM_000021.3), confirmed by Sanger sequencing (Figure 1B). The c.871A > C (p.Thr291Pro) is a known pathogenic missense mutation located in exon 9.²⁶ The p.Thr291Pro has an anti-parallel β -sheet location, which, according to recent structural data, interacts directly with the C-terminal domain of the substrates (APP and Notch).^{27,28}

3.2 | The p.Thr291Pro variant leading to generation of relatively longer A β peptides from APP

To investigate whether the pathogenic variant identified in our cohort, the p.Thr291Pro *PSEN1* substitution, destabilizes the interactions between γ -secretase and APP leading to altered A β production, we analyzed the activities and thermostabilities of WT and mutant γ -secretase complexes *in vitro*. To this end, we rescued the expression of human WT and p.Thr291Pro mutant *PSEN1*s in *PSEN1/2*-deficient mouse embryonic fibroblasts (MEFs) and analyzed the effects of the mutation on γ -secretase complex reconstitution and activity. The WT and mutant *PSEN1* proteins both restored γ -secretase complex levels, as indicated by the levels of NTF- and CTF-*PSEN* fragments (Figure 2A). We next assessed the effects of the p.Thr291Pro substitution on the generation of A β peptides in an *in vitro* γ -secretase activity assay. The analysis showed increased production of A β 42, relative to the shorter A β 40/38 peptides by the mutant protease when compared to the WT. The increased production of A β 42, relative to the shorter A β 40/38, is a hallmark of familial AD-linked *PSEN1* mutations. Analysis of the p.Thr291Pro *PSEN1* in thermoactivity assays confirmed that

this substitution destabilizes “enzyme-substrate” interactions at the level of both total activity (AICD generation) as well as A β production (Figure 2B-2C). This is indicated by shifts in the thermoactivity profiles for AICD and A β peptide production, respectively. The lower stability of enzyme-substrate interactions formed by the p.Thr291Pro *PSEN1* γ -secretase is in line with the reported effects of AD-linked *PSEN1* variants on APP processing.^{16,17,18,29} We point at this effect as central to the pathogenesis of AD-linked *PSEN1* variants.

3.3 | Spastic paraplegia phenotype in *PSEN1*-related AD

Given the unusual presentation of pure SP preceding cognitive impairment by several years, we reviewed the clinical phenotype of all known *PSEN1* pathogenic variants reported to date. We found that 13.7% of reported pathogenic variants were associated with SP (31 of 226 pathogenic and likely pathogenic variants reported in *PSEN1*) (<http://www.molgen.ua.ac.be/ADMutations>). Of these, including the variant reported here, 17 (7.5%) have initially presented with pure SP, followed by cognitive decline months or years later (Table 1, Figure 1C). The average age at onset in the group of patients presenting initially with pure SP was 40.5 years old, and the majority of these variants were located in or after exon 8. The average age at onset in the *PSEN1* mutation carriers manifesting only with dementia was 37.3 years old and SP was, in most of these cases, prominent only after some years of cognitive impairment (Supplementary e-2). The majority of patients with this phenotype had mutations that clustered in exons 4 and 5¹.

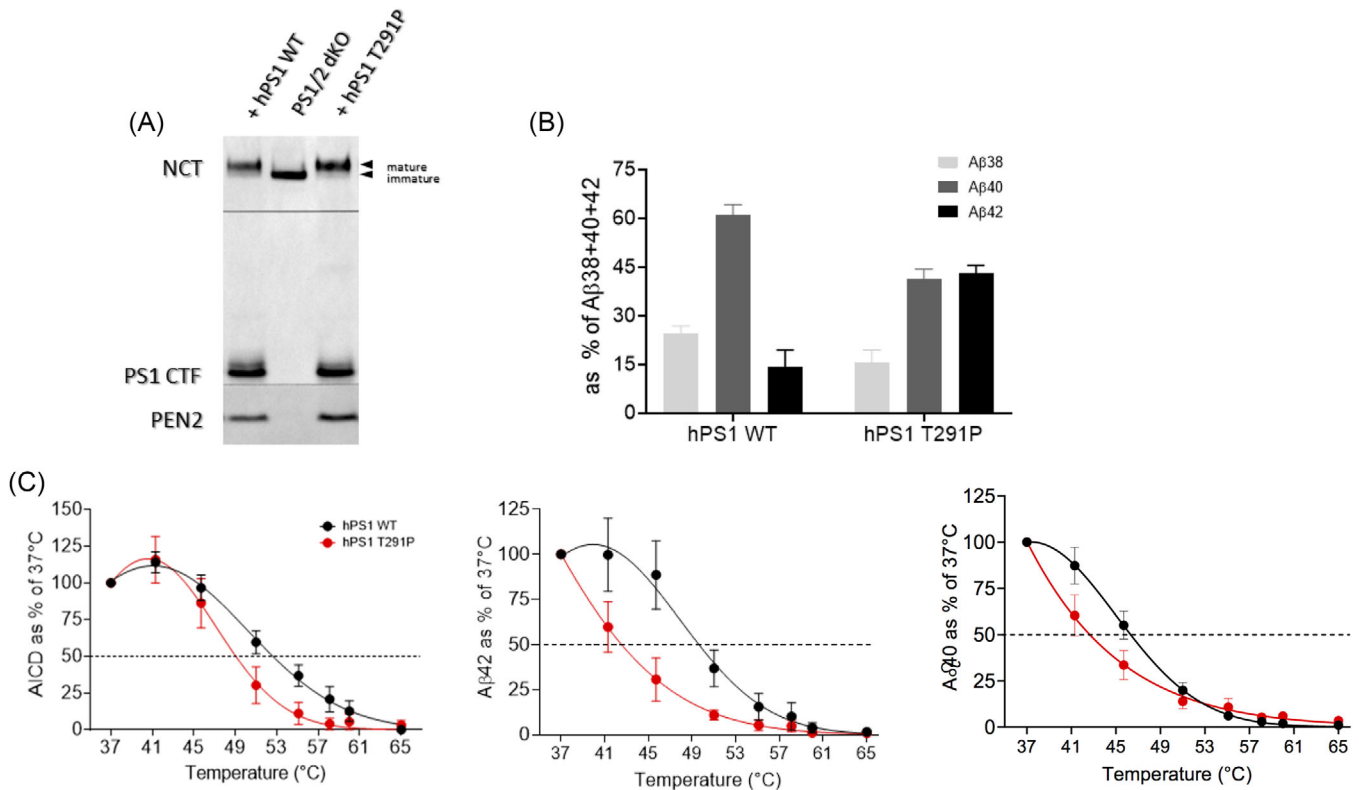


FIGURE 2 AD-linked p.Thr291Pro PSEN1 destabilizes γ -secretase-APPC99/ $A\beta$ interactions resulting in generation of longer $A\beta$ peptides. A, Representative SDS-PAGE/western blot analysis of CHAPSO-solubilized membranes from dKO PS1/PS2 mouse embryonic fibroblasts (MEFs) stably expressing wild-type (WT) or mutant p.Thr291Pro hPS1. The presence of mature, glycosylated NCT, C-terminal fragment of the endoproteolyzed PS1 and PEN2 (compared to dKO PS1/PS2 cells) indicates that p.Thr291Pro hPS1 reconstitutes active γ -secretase complexes. Arrowheads indicate the position of molecular weight markers. B, $A\beta$ profiles generated by solubilized membranes prepared from stable MEF PS1/2 dKO cell lines expressing WT or p.Thr291Pro hPS1 γ -secretase in vitro activity assays with purified APPC99-3xFLAG. Increase in the production of $A\beta_{42}$ at the expense of the shorter $A\beta_{38}$ and $A\beta_{40}$ indicates pathogenic impairment of γ -secretase processivity of APPC99 by the AD-linked p.Thr291Pro hPS1 mutation. $N = 3 \pm SD$. C, In vitro thermoactivity data confirm that mutant p.Thr291Pro hPS1 destabilizes γ -secretase-APPC99/ $A\beta$ interactions, when compared to the WT complex, at the level of total activity (AICD generation) as well as $A\beta$ peptide production, inducing premature dissociation of long $A\beta$ peptides. $N \geq 3 \pm SD$

3.4 | Hereditary spastic paraplegia pathways including PSEN1 act as a true interactome

Given the occurrence of SP phenotype in the early course of *PSEN1*-related AD in over 10% of all reported cases, we investigated the overlap between HSP and *PSEN1* dysfunction using gene set enrichment analysis (GSEA) and *in silico* knockdown of *PSEN1* from the resulting network (Supplementary e-1). HSP-associated genes were significantly enriched for gene ontology terms and pathways involving neurogenesis, axonal development, and CNS myelination, indicating a point of divergence from the rest of the interactome. It is important to note that the retrieval of significantly enriched pathways (particularly by examining the gene list containing *PSEN1*) has indicated that the HSP-causative genes function as a true interactome. Following *PSEN1* knockdown, the pathways “neurotrophin signaling” and “Wnt signaling” were eliminated. In canonical (β -catenin dependent) Wnt signaling, presenilins maintain β -catenin stability, allowing the translocation to the nucleus and the subsequent expression of Wnt target genes, promoting neuronal growth, synaptogenesis, and plasticity.

Much like the perturbed Wnt signaling disrupts adult neurogenesis and plasticity in AD, deleterious alterations have also been reported as upstream events in neurotrophin cascades³⁰ (such as BDNF signaling). *In vivo* animal models of *PSEN1* knockdown have demonstrated the conditional depletion of BDNF levels, corresponding to the pathway silencing denoted in our *in silico* experiment.

3.5 | Neuropathological findings in PSEN1 variants presenting with spastic paraplegia show distinct features from sporadic AD: Case report and extended analysis

Although the *PSEN1* c.871A > C was reported previously in familial AD, its neuropathological expression has not been previously described. The brain biopsy in individual II-1 from this study showed amyloid deposition within the vessel walls with diffuse cortical plaques, with the characteristic morphology of CWPs (Figure 3). Neuropathological studies of 24 mutations of *PSEN1*-related familial AD associated with

TABLE 1 All published *PSEN1* mutations presenting with pure SP at onset

Mutations	Exon	Families (n)	Age of onsetSP (years)	Neuropathological findings	Presenting symptom	Study
p.Ile83_Met84del	EX4	1	34-38	CWP	SP	Houlden et al. (2000)
p.Tyr154Asn	EX5	1	37	-	SP	Hattori et al. (2004)
p.Phe237Ile	EX7	1	31	-	SP	Sodeyama et al. (2001)
p.Gln223Arg	EX7	1	35	CWP, neuritic plaques, neurofibrillary tangles, neuropil threads	SP	Saint-Aubert et al. (2013)
p.Val261Phe	EX8	1	38	CWP	SP	Farlow et al (2000)
p.Pro264Leu	EX8	1	54	-	SP	Jacquemont et al. (2002)
p.Pro264Leu	EX8	1	-	-	SP	Dumanchin et al. (2006)
p.Pro264Leu	EX8	1	-	CWP	SP	Wallon et al. (2012)
p.Arg278Thr	EX8	1	34	-	SP	Kwok et al. (1997)
p.Arg278Lys	EX8	1	41-45	-	SP	Assini et al. (2003)
p.Glu280Gly	EX8	1	40-43	Amyloid plaques, CWP	SP, myoclonus, ataxia	O'Riordan et al. (2002)
p.Glu280Gly	EX8	1	47	Amyloid plaques, CWP, tau positive neurofibrillary tangles	SP	Sinha et al. (2013)
Skipping of entire exon 9	-	2	45-55	CWP Neurofibrillary tangles, amyloid plaques	SP	Crook et al. (1998) Brooks et al. (2003)
p.Thr291Pro	EX9	2	32	CWP	SP	This study
p.Thr291Pro	EX9	1	33	-	SP	Dumanchin et al. (2006)
p.Val391Gly	EX11	1	23	-	SP	Lou et al. (2017)
p.Pro436Gly	EX12	1	20-42	CWP	SP	Houlden et al. (2000)

Legend: SP, spastic paraparesis; CWP, cotton-wool plaques; -, not available; EX, exon. The novel *PSEN1* case (p.Thr291Pro) identified in this study is also listed.

SP were identified in the literature. CWPs were identified in 21 of 24 studies, amyloid plaques in 10 of 24, and cerebral amyloid angiopathy (CAA) in 3 of 24. Different amounts of CAA in different mutations were also observed. In mutations located before codon 200, many diffuse cored plaques, a few white matter plaques, and mild to moderate CAA were identified. In comparison, mutations beyond codon 200 showed larger diffuse, cored plaques surrounding with amyloid-rings arteries, with severe CAA (Table 1).

4 | DISCUSSION

In this study, we assessed the clinical, molecular, and neuropathological features of AD-linked *PSEN1* mutations presenting with pure HSP-like phenotype several years before the onset of cognitive decline. Our analysis of all reported AD-linked *PSEN1* cases showed that 7.5% of all known pathogenic *PSEN1* pathogenic variants presented initially as SP. Furthermore, 13.7% of *PSEN1* pathogenic variants were associated with SP at some point during the disease, suggesting that this phenotype is not infrequent and should prompt inclusion of *PSEN1* variants in the differential diagnosis of patients presenting with SP.

We show that atypical presentations including SP are mostly associated with *PSEN1* variants located beyond exon 8. Pathogenic variants located before codon 200 in exon 8, affecting the first hydrophilic loop of *PSEN1*, usually present with an earlier age of onset and pure dementia phenotype,¹ whereas the *PSEN1* cases manifesting initially as SP have a later age at onset, in the same range with HSP patients, making the differential diagnosis of familial dementia challenging.

Our study, the first neuropathological description of AD caused by p.Thr291Pro *PSEN1* variant, shows A β -A4-deposition within the vessel walls with several diffuse CWPs. We cannot comment further as the patient is alive, and no full autopsy report is available. Neuropathological studies of *PSEN1* families presenting with SP identified CWPs, consisting of congophilic cores and dystrophic neurites, which were not observed in sporadic AD,^{19,20} but are seen in other familial AD cases, such as *PSEN2* or *APP*-related. CWPs consist mainly of aminotermally truncated forms of A β peptide, starting after amino acid 11, where the alternative cleavage site is located.¹³ *PSEN1* mutations with CWPs produce high levels of the A β peptide ending at amino acid 42 or 43.¹³ CWPs are found mainly in the frontal motor cortices. However, abnormal tau accumulation in the primary motor cortex was reported recently in a *PSEN1* patient with SP.³¹ Neurofibrillary tangles (NFTs)

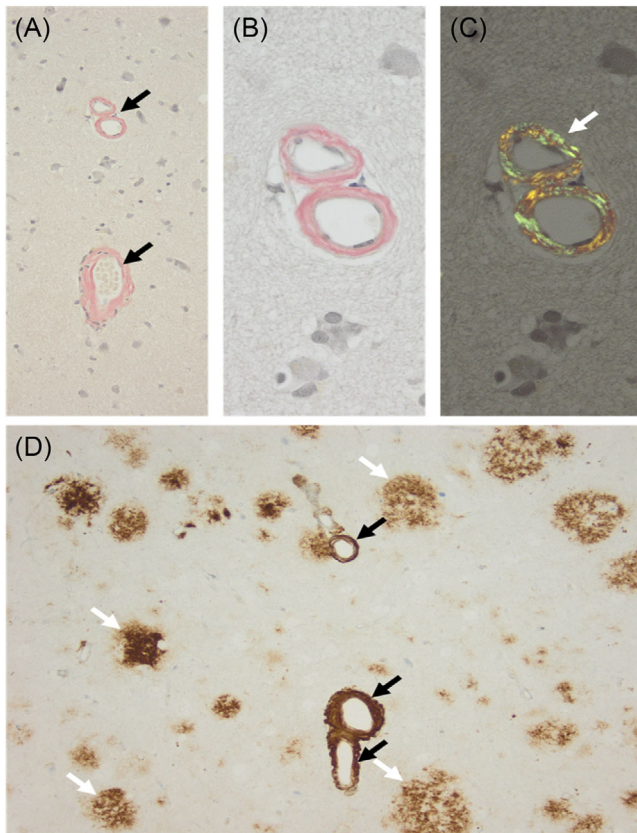


FIGURE 3 Neuropathological study in individual II-1 from this study (c.871A \geq C, p.Thr291Pro). A, Several vessels with thickened vessels showing congophilia (black arrows; Congo Red staining). B, Higher magnification of A with congophilia of blood vessels (B) and after polarization of the same area showing typical apple-green color (white arrow in C). D, Beta-A4-amyloid immunohistochemistry confirms amyloid deposition within the vessel walls (black arrows) and in addition several diffuse cortical plaques (white arrows), with the characteristic morphology of cotton-wool plaques

were identified in several cases carrying *PSEN1* variants and manifesting SP.^{26,32} However, not all *PSEN1*-related cases associated with SP showed the presence of overt tangles.³³ On neuropathology examination, *PSEN1* mutations located beyond codon 200, as in the case presented here, have more extensive CAA than mutations located before codon 200.³⁴

Pathway analysis in HSP-related genes and *PSEN1* showed perturbed “neurotrophin signaling” and “Wnt signaling” when *PSEN1* is knocked down. Both these pathways have been associated with the pathogenesis of AD, mediating both neuronal morphogenesis and mature CNS neuroprotection. Furthermore, the roles mediated by these pathways include motor neuron development, phenotype diversification, and survival.^{30,35} Conversely, loss of canonical signaling in each of these pathways has been implicated in the pathophysiology of ALS.³⁶ Restoration or de novo targeting of these pathways, in turn, has been shown to either halt neuron death or promote proliferation.³³ Taken together, these studies suggest that a knockdown in either or both of these pathways could promote neurodegeneration and synap-

topathy, and that the arrest of these aberrant processes could occur if canonical signaling were restored. Thus pathways and molecular functions mediated by this interactome would further outline potentially targetable pathophysiological mechanisms.

The frequent co-occurrence of *PSEN1* variants and spasticity might thereby be driven by shared vulnerability of corticospinal tract axons and cortical neurons toward disturbances of the same molecular pathways and the effect of specific mutations affecting γ -secretase activity in a way that alters its catalytic action on some other substrates particularly important for motor neurons. Over 80 different γ -secretase substrates have been identified³⁷ and a growing number of additional proteins cleaved by presenilin/ γ -secretase continue to be discovered. Most of these proteins are type-I transmembrane proteins involved in vital signaling functions regulating cell fate, adhesion, migration, neurite outgrowth, or synaptogenesis. It is notable that the HSP networks analyses highlight very similar molecular disease mechanisms including cellular transport, nucleotide metabolism, and synapse and axon development.³⁸ Furthermore, some presenilin γ -secretase substrates have already been linked with neurodegenerative conditions associated with spasticity and/or motor neuron involvement such as colony-stimulating factor-1 (CSF1R),^{39–41} netrin 1 receptor (DCC),^{42,43} receptor tyrosine-protein kinase erbB-4 (ERBB4),⁴⁴ and very low density lipoprotein receptor (VLDLR).^{45,46}

Two distinct pathogenic pathways emerge in *PSEN1*-related disease. First, the typical AD type presenting initially with dementia and characterized by neuritic plaques on brain biopsy, and the atypical course presenting initially with SP followed by cognitive decline and characterized by CWP on neuropathology.²⁰ A “threshold effect” was speculated (which most *PSEN1* mutations fail to exceed) as well as a differential effect on $A\beta$ 42 production.^{20,26} Some mutations are reported to cause a nearly complete loss of γ -secretase activity, suggesting that pathogenic *PSEN1* mutations could also cause a general loss of presenilin function.⁴⁷ However, the great majority of familial AD patients are heterozygous for *PSEN1* mutations and the studies analyzing γ -secretase activity in post-mortem brain samples of different *PSEN1*-related familial AD show that the WT allele not only contributes to the global brain γ -secretase endopeptidase activity but in fact compensates for potential mutant-associated impairments, at least on APP processing.¹⁷ Mutant-induced impairments on the γ -secretase-processing of particular substrates cannot be excluded.

$A\beta$ 42/ $A\beta$ 40 ratio and increased percentage of N-terminal truncated species are also speculated to contribute to these phenotypic differences,⁴⁸ suggesting the existence of critical areas of the protein. For example, mutations in exons 4 and 5 are reported to cause, particularly, early onset disease. Without affecting the endopeptidase function of γ -secretase, these mutations located in the extracellular loop change an important allosteric core that alters the $A\beta$ profiles generated through carboxypeptidase-like activity of γ -secretase.²⁴ Our analyses show that the identified p.Thr291Pro *PSEN1* mutation does not impair the assembly of γ -secretase, or its activation through autoendoproteolysis into an active protease. The mutation, however, destabilizes the γ -secretase-APP/ $A\beta$ interactions, leading to premature dissociation of longer $A\beta$ peptides. This destabilizing effect has been

shown to characterize AD-linked *PSEN1* mutations and correlates with AD onset.¹⁸

Compared to *PSEN1*-associated SP, HSP usually has a slow progression and does not shorten patients' life expectancy; therefore, a definite molecular diagnosis of familial *PSEN1*-related disease has major implications for adequate counseling, healthcare provision, and access to treatments and services. We highlight the urgency to screen these patients for *PSEN1* mutations, especially in the age of emerging anti-amyloid disease-modifying therapy. A delay in the treatment of familial AD, often by years, because of an initial presentation as HSP, could have a significant negative impact on the patient and their family. Therefore, we recommend that the mutations in *PSEN1* should be considered in the genetic work-up of all patients presenting with SP, especially when associated with cognitive impairment or in those with a family history of early onset dementia; and that, consequently, *PSEN1* should be included in HSP diagnostic gene panels.

Our study highlights the clinical overlap between SP and *PSEN1*-related AD. We show that over 7% of *PSEN1* mutations present with pure HSP phenotype preceding onset of dementia by several years. These mutations were associated with cortical CWP on neuropathological examination. Early screening of *PSEN1* mutations would alleviate the need for further invasive testing, such as brain biopsy. Unraveling the phenotypic heterogeneity within *PSEN1* mutation carriers could provide new potential targets for clinical trials of patients with familial AD.

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CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

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